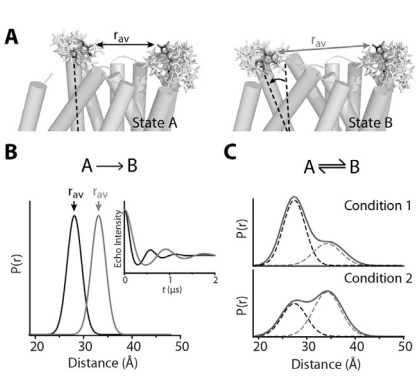
2303-Wkshp
Domains in Supported Bilayers: from Winemaking to Protein Nanopatterning
Marjorie L. Longo.
UC Davis, Davis, CA, USA.
Applications of phase-separated domains in supported bilayers will be discussed including: measuring line tension, domain growth kinetics, anomalous diffusion, ternary phase diagrams, and curvature patterning of lipids and proteins. Supported bilayers were used in these studies because they are amenable to high resolution imaging and patterning techniques such as atomic force microscopy, single molecule tracking, electron beam lithography, and layered deposition techniques (e.g. spin coating). We were able to record time-dependent and metastable behavior of phase-separated/separating systems using these techniques to determine parameters, and explore dynamic and kinetic regimes. These measurements and analysis may have potential interest in the study of membrane rafts, cell membranes of wine yeasts, crowded cell membranes, curvature variant membranes of cellular organelles, and the engineering application of supported bilayers.

Workshop: Distance Measurements by Double Electron Electron Resonance (DEER)

2304-Wkshp
DEER on Nitroxides: Experiment and Data Interpretation
Yevhen Polyhach.
Swiss Federal Institute of Technology, Zürich, Switzerland.
By measuring distances and distance distributions between electron spins with the Double Electron Electron Resonance experiment a number of questions on structure and function of biological systems can be answered. The method has gained its high popularity due to a relatively moderate experimental effort combined with a good precision and reliability of information on a nanometer distance range that it can provide. Very often DEER is used together with the site-directed spin labelling (SDSL). In such a way spin markers (labels) are introduced at desired positions of a paramagnetically silent protein and interspin distances are monitored.
In the presentation, key moments important for understanding the DEER experiment, conducting it at optimal conditions and performing reliable extraction of distance information are discussed. To this end, attention is given to rather general questions (pulse sequence, distance range, sample concentration etc.) as well as to very specific issues of the background correction and the distance extraction.
The size of common spin labels is comparable with the distance range of the DEER experiment. Additionally, the conformational flexibility of the labels is also high. Therefore making reliable quantitative conclusions on a protein in hands is hardly possible if spin label is not taken into account during the analysis of the DEER data. A method of doing so based on the rotamer library approach is discussed.
The presentation will be largely based on the open-source software packages Deeranalysis and MMM.

2305-Wkshp
Mapping Transporter Conformational Dynamics using Double Electron Electron Spectroscopy (DEER)
Hassane S. Mchaourab, Ph.D.
Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA.


Active transporters couple the energetically uphill transport of substrate against their concentration gradients to direct use of ATP energy or the discharge of electrochemical ion gradients. Mapping the conformational motion that transduces the energy input into the work of substrate translocation is central to understanding transport mechanism. Spin labeling and electron paramagnetic resonance (EPR) spectroscopy offers a unique window into membrane protein conformational dynamics in the native-like environment of a lipid bilayer. Specifically, Double Electron-Electron Resonance (DEER) spectroscopy enables long range distance measurements between two spin labels yielding a distance distribution. The amplitude of movements can be determined from changes in the average distance while the width of the distance distribution can be interpreted in terms of underlying transporter conformational equilibria. We have applied spin labeling and DEER to define the proton-activated conformational switch of the multidrug antiporter LmrP, to uncover asymmetric conformations of the ABC heterodimer BrmCD, and to test models of the Na-symporter LeuT. These examples illustrate the importance of spectroscopic approaches for bridging structure and mechanism for membrane proteins.

2306-Wkshp
Evaluating DEER Distance Profiles in Terms of Protein Conformational Ensembles
Xi Huang, Ian S. Mitchell de Vera, Mandy E. Blackburn, Luis Galiano, **Gail E. Fanucci, PhD**.
Chemistry, University of Florida, Gainesville, FL, USA.
Distance profiles obtained from DEER spectroscopy provide a wealth of information regarding distances between spin labels in macromolecular systems. We have been evaluating the utility of DEER distance profiles to provide information regarding changes in conformational sampling ensembles in HIV-1 protease. In this workshop we will present our approaches and developments related to assessing errors in the data and how the data are related back to protein conformational sampling states. We will also show that sample freezing methods do not alter HIV distance ensembles within errors of the measurements.

2307-Wkshp
DEER Studies of Membrane Proteins
Gary A. Lorigan, Ph.D.
Chemistry and Biochemistry, Miami University, Oxford, OH, USA.
Pulsed Electron Paramagnetic Resonance (EPR) spectroscopic techniques such as Double Electron-Electron Resonance (DEER) can provide pertinent structural information on a wide variety of biological systems that have been spin-labeled. This powerful technique can be used to measure distances between 2 spin labels out to about 70 Å. However, the application of DEER spectroscopy to study membrane proteins can be difficult due to short phase memory times (Tm) and weak DEER modulation in more biologically relevant proteoliposomes when compared to water soluble proteins or membrane proteins in detergent micelles. The combination of these factors often leads to broad distance distributions, poor signal to noise, and limitations in the determination of longer distances. The short phase memory times are typically due to uneven distributions of spin-labeled protein within the lipid bilayer, which creates local inhomogeneous pockets of high spin concentrations. Approaches to overcome these limitations and improve the quality of DEER measurements for membrane proteins will be discussed: lipodisq nanoparticles, bi-functional spin labels (BSL), and Q-band pulsed EPR spectroscopy.

2308-Wkshp
Do Spin Labels Tell the Truth?
Peter Fajer, Fajer Mikolai, Michael Zawrotny, Wei Yang.
Florida State University, Tallahassee, FL, USA.
We have developed general strategies for simulation of rotamer structures of spin labels. The underlying criteria for those simulations are: (a) ***exhaustive sampling*** of rotamer space; (b) ***consensus*** of results independent of rotamer starting points.
Those two criteria can be satisfied only when the number of transitions in any dihedral angle exceeds 100. Consensus of 18-27 different starting points is necessary to ascertain extensive sampling. Current methods such as MD are not sufficient to overcome barriers ~50-200kcal . Simulated annealing designed to overcome such barriers suffers from large fluctuations of the protein background. Monte Carlo ignores the entropic effects and suffers from fixed protein environment. Simulated Scaling method, avoids those problems modulating the electrostatic and dihedral energies between 0 (to allow for traversing energy barriers, maxima) and full potential (sampling minima). The enhanced simulation is applied to a label and to the immediate (<10 Å) protein environment. Simulations show: (a) labels adopt 2-4 equally populated rotamers, single conformation is rarely observed; (b) position of the NO varies up to 12 Å for a single site; (c) the disorder is not a uniform distribution but punctuated maxima separated. (d) different rotamers are separated by energetic barriers to large to be motionally averaged (> 30kcal).
These results illustrate necessity for caution when interpreting EPR signals in terms of molecular structure or behavior. For example the 12 Å distance change in DEER spectra should not be interpreted as a large conformational change, it can well be a flip of a spin label about Cα -Cβ bond. **Rigorous exploration of possible rotamer structures of a spin label is paramount in signal interpretation**.